ABSTRACT

Charles University in Prague

Faculty of Pharmacy in Hradec Králové

Department of Pharmacology and Toxicology

Candidate: Lucie Sedlářová

Supervisor: Dr. Přemysl Mladěnka, Ph.D.

Title of thesis: Standardization of method for in vitro assessment of copper chelation.

Cooper is a trace element, which is essential for many biochemical processes in the human body. Disruption of copper homeostasis can cause a variety of diseases. There are primarily two hereditary diseases associated with copper dyshomeostasis: Wilson´s disease, which is linked with the excess of copper in the liver and other tissues, and Menkes disease, which is associated with a lack of systemic copper. In addition, local disturbances of copper play a role also in other diseases (neurodegenerative diseases, tumors, myocardial infarction).

The main objective of this experimental work was the standardization of a simple but accurate method for rapid screening of copper chelators, which could be applied for the therapy of systemic or local copper excess in the future.

The methodology is based on the spectrophotometric determination of copper in microplates using appropriate indicators, bathocuproindisulfonic acid disodium salt (BCS) and hematoxylin.

This thesis has shown that the BCS methodology is able to determine chelation of cupric as well as cuprous ions at four different pH (4.5, 5.5, 6.8, 7.5) corresponding with physiological or pathophysiological conditions of the organism. In addition to the assessment of chelating properties, this methodology can be used for the determination of reducing properties of tested compounds. Hematoxylin can be used only for the assessment of the

chelation of cupric ions and only at higher pH. Performed methodologies were successfully standardized particularly in relation to the optimal amount of reactants and applied in chelation experiments with a known chelating compound clioquinol.

The developed methodology represents very simple and accurate way to measure copper chelation.

KEYWORDS:

Copper, Chelator, Bathocuproine, Hematoxylin